The University of Chicago

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A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY
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RESPONSES OF PTERIS LONGIFOLIA TO APPLICATIONS OF AMMONIUM 2,4-DICHLOROPHENOXYACETATE[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 575

BERNARD STRICKLER

Introduction

Studies of the effects of 2,4-dichlorophenoxyacetic acid on plants with but one exception (16) have been confined to the responses of various angiosperms. Prior investigations have tended largely toward the refinement of techniques of applying this substance (17, 12) or an extension of its practical uses (2, 11, 13). The selective sensitivity to 2,4-dichlorophenoxyacetic acid of different species of plants, combined with the resultant telemorphic effects (3), has been of distinct advantage in its use as a herbicide.

In the present experiment information on the action of 2,4-dichlorophenoxyacetic acid is extended to the pteridophytes, especially to the gametophytic generation. The responses of the prothallia suggest the possibility of utilizing this compound as a tool of experimental morphology.

Material and methods

Spores were collected from a single leaf of *Pteris longifolia* grown in the greenhouses of the University of Chicago. Germination and subsequent culture of the gametophytes were carried out on new $2\frac{1}{2}$-inch unglazed clay pots which had been washed and then fired in an electric muffle furnace. The pots were then packed with white quartz sand which had previously been washed with distilled water and autoclaved. They were then inverted in small preparation dishes to which various solutions were added. When the entire pot became wetted, spores were sown on the topmost surface by tapping them from a dissecting needle. Enough solution was then added to submerge just the rim of the pot. The solutions were maintained at this level throughout the experiment by the addition of distilled water. The cultures were covered with transparent, untinted, cylindrical battery jars.

All solutions consisted basically of a one-half dilution of ZINZADZÉ's slightly acid formula (18), pH 6.1. Using this diluted nutrient solution, a stock solution containing 100 mg./l. of the ammonium salt of 2,4-dichlorophenoxyacetic acid was prepared. The ammonium salt was prepared by dissolving 2,4-dichlorophenoxyacetic acid in 95% ethyl alcohol, bubbling anhydrous ammonia gas into the above solution, washing the precipitate with absolute alcohol, and then drying. The final concentrations of the salt used in the experimental series were 50, 10, 1, and 0 mg./l.

The gametophytes received 10 hours of illumination daily from six 36-inch, 30-watt Mazda daylight fluorescent tubes set in a reflector (fig. 1). The temperature of the laboratory in which the prothallia were grown ranged approximately from 75° to 85° F.

Sporophytes used in the experiments were of two ages: small, young plants grown in pots, and large, mature plants growing undisturbed on a soil and rock substrate in the greenhouse. Their petioles and terminal pinnae were treated

[1] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

with concentrations of 2,4-dichlorophenoxyacetic acid ranging from 125 to 2000 p.p.m. These were applied either as lanolin mixtures of the acid or as aqueous solutions of the ammonium salt with Duponol C (9) as a spreader.

Gametophyte and sporophyte material prepared for microscopic examination was fixed in formalin-aceto-alcohol. Whole mounts of the prothallia were stained in Harris' haematoxylin, counterstained in orange G, and mounted in balsam. Sectioned material of the prothallia and sporophytes was prepared according to the tertiary butyl alcohol–paraffin method and stained with either the safranin–fast green combination or Flemming's triple stain.

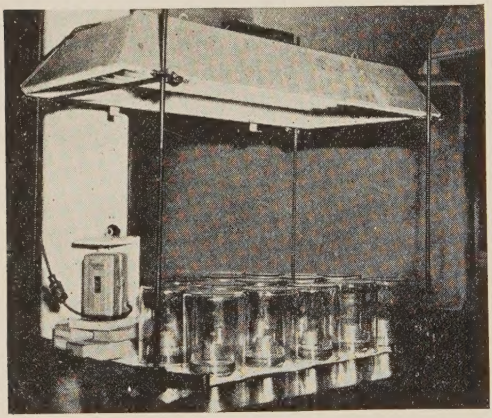


FIG. 1.—Arrangement of cultures of gametophytes under fluorescent lights.

Observations

SPORE GERMINATION

Throughout the course of the studies on the gametophytes, there was a high percentage of germination in all cultures. Nevertheless, in those cultures containing 10 and 50 mg./l. of ammonium 2,4-dichlorophenoxyacetate, a larger number of ungerminated spores were conspicuously present than in those containing plain ZINZADZÉ's solution or only 1 mg./l. of the salt. This led to the idea that the presence of this substance at the higher concentrations may have significantly reduced the percentage of germination. The larger size attained by the prothallia in the most dilute and control cultures, however, may have merely obscured the ungerminated spores, so that the visible differences might be more apparent than real.

To test this idea, a series of cultures was set up, according to the methods previously described, in which a number of 5 mm. squares were scratched into the surface of the pots on which the spores were to be planted. In each culture a combined total of 1000 ungerminated and germinated spores was counted. By confining the counts only to spores lying within the squares, the manner of selecting those to be counted was the same for all cultures.

In connection with the standardization of data on the percentage of germination, it should be noted that the significance of such data is affected by the differences in concepts of the definitive stage of spore germination. For example, ORTH (14) designated as germination that stage in development after the splitting of the exospore in which the first rhizoid or some other growth process is evident and chlorophyll is formed. Data based on counts of spores at earlier stages of development would fail in meaning for those who subscribe to ORTH's designation, since the number of plants which attain the requisite stage would be unknown.

Another factor of importance for this type of data is the time interval at which counts are made. DOPP (5) pointed out that the spores of a species of fern, even within the same culture, do not germinate at the same time. The successive

germination of spores of *Pteris longifolia* can be illustrated from our experiment, in which a preliminary count of 600 spores in a control culture revealed that the primary cell had emerged in 244 spores by the seventh day after sowing. Another count of the same area 2 days later showed 419 spores in the same or later stage of development.

For these reasons counts were made 10 days after the emergence of initial prothallial cells was first observed. All counts were made on the same day. Germinated spores then possessed more than one rhizoid and a number of prothallial cells. Ungerminated spores had failed even to split the exospore. The uniformity of germination among all the cultures (table 1) does not support the idea that germination was retarded either in extent or in time by the presence of ammonium 2,4-dichlorophenoxyacetate.

TABLE 1

RESULTS OF TESTS OF GERMINATION OF SPORES OF PTERIS LONGIFOLIA IN AMMONIUM 2,4-DICHLOROPHENOXYACETATE

Concentration of salt (mg./l.)	Ungerminated spores	Germinated spores	Total	Percentage of germination
0	257	748	1005	74.4
1	252	753	1005	74.9
10	247	756	1003	75.3
50	254	773	1027	75.1

GAMETOPHYTE

Growth of the gametophytes was observed from whole mounts of prepared and fresh material collected at 3- or 4-day intervals. All stages of the treated plants were compared with untreated prothallia collected at the same times. Responses of gametophytes to the three concentrations of ammonium 2,4-dichlorophenoxyacetate used—though seemingly quite different at each concentration—showed two types of reactions which were fundamentally the same in all treated cultures: (*a*) an inhibition of growth in size and (*b*) abnormal patterns of development.

INHIBITORY EFFECTS.—A difference in size was noted in gametophytes which were only 3 or 4 days old (figs. 2, 3). At this stage most plants possessed only one rhizoid; a few had two. At concentrations of 50 and 10 mg./l., the gametophytes were comprised of the single rhizoid and one or two prothallial cells, while those at 1 mg./l. and the control plants had filaments four or more cells long. As already mentioned, spores placed in nutrient solutions containing ammonium 2,4-dichlorophenoxyacetate were not delayed in germination so that this difference cannot be ascribed to age. That such is not the case is obvious from observations made on older gametophytes. Thirteen-day-old gametophytes at 50 mg./l. were still filamentous, consisting of about seven prothallial cells and about six rhizoids (fig. 4). At all other concentrations the gametophytes were thalloid; the smallest being those grown at 10 mg./l.—the largest, the control plants (figs. 5, 6, 7). By the 27th day the untreated gametophytes had formed the typical heart-shaped prothallium; those at 1 mg./l. had begun to form a second lobe; the gametophytes at 10 mg./l. consisted of a single lobe, with no indication of forming a second one, while those at 50 mg./l. had remained essentially filamentous except for a small amorphous mass of cells near the apex.

Measurements of gametophytes when they were 3 and 27 days old showed that the size of prothallia grown at 50 mg./l. had increased only 3.39 times; at 10 mg./l., 48; at 1 mg./l., 113; while the control prothallia had increased 180

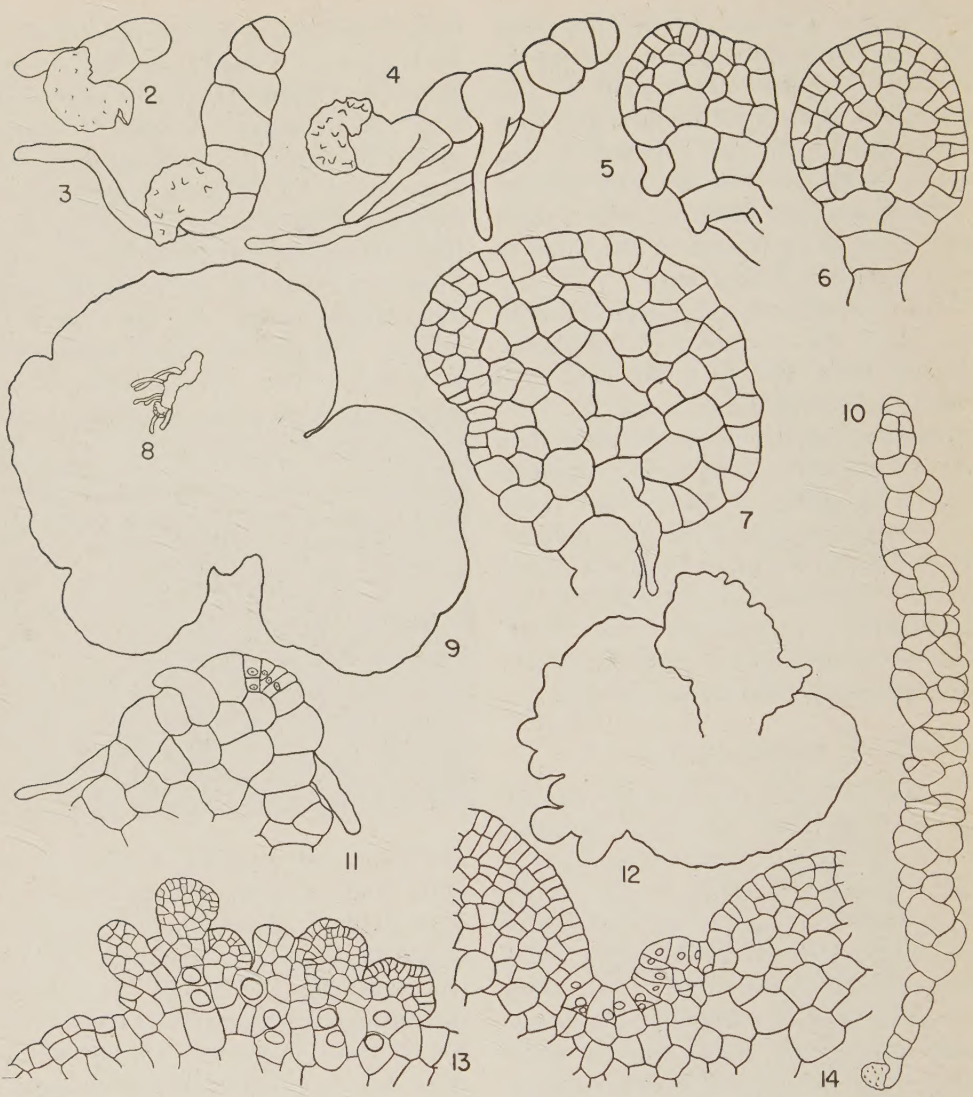


FIG. 2–14.—Gametophytes grown on various concentrations of ammonium 2,4-dichlorophenoxyacetate in ZINZADZÉ's nutrient solution (rhizoids mostly omitted). Figs. 2, 3, gametophytes 3 days old grown at 50 and 0 mg./l., respectively. Figs. 4–7, relative size of gametophytes 13 days old grown at 50, 10, 1, and 0 mg./l., respectively. In fig. 7 the meristem primordium is seen in typical lateral position on first lobe. Figs. 8, 9, relative size of gametophytes 30 days old at 50 and 0 mg./l., respectively. Fig. 10, gametophyte grown for 4 months at 50 mg./l., showing hemispherical end cell and juvenile filamentous condition. Fig. 11, apical portion of prothallium 27 days old, grown at 10 mg./l., with apical position of meristem on single thallus lobe. Fig. 12, prothallium 51 days old, at 1 mg./l., with vertical third wing and secondary prothallia on basal portion of thallus. Fig. 13, details of secondary prothallia of fig. 12. Fig. 14, early stage in development of third wing from base of sinus.

times. These values are based upon measurements of camera lucida drawings of the surface areas of the gametophytes. Each value represents the average of several gametophytes.

ALBAUM (1) has shown that, for fern prothallia composed essentially of a single layer of cells, surface area is a fair index of the mass of tissue present at any time and that increase in surface area may thus be used as an index of growth. Beyond the 27th day the prothallia in the present experiment developed more than a single layer of cells. After this, surface area was not used as an index of growth.

Differences in size between gametophytes of the same age grown at different concentrations of the salt were due chiefly to differences in cell number. The largest cells in the treated prothallia (figs. 4, 5, 6) were equal in size to those of untreated prothallia of the same age (fig. 7). This indicates that the two growth phases—cell division and cell enlargement—were dissimilarly affected by the experimental solutions. This is clearly shown in the prothallium in figure 5. Here the small number of larger, central cells composing the bulk of the thallus are surmounted by a marginal row of smaller cells of similar size, the "Randmeristem" described by ORTH. Intermediate-sized cells (fig. 7), which normally lie between the marginal meristematic cells and the more mature basal and central cells, are for the most part lacking, indicating that cell division but not cell enlargement was inhibited.

Striking evidence of the inhibitory action of the salt is seen in the comparison of 30-day-old gametophytes grown at a concentration of 50 mg./l. with control prothallia of the same age (figs. 8, 9).

FORMATIVE EFFECTS AT 50 MG./L.—There was no evidence of plate formation in gametophytes grown at 50 mg./l. until 30 days after germination. Until this time they consisted of a linear series of cells always derived from divisions of the hemispherical terminal cell. The filament was distorted, owing to the unequal distension and consequent bulging of the wall of each maturing cell. Except for the formation of rhizoids, divisions of these derivatives were rarely seen. After the 30th day subsequent derivatives of the end cell divided in an irregular manner. Ultimately, an elongated clump of distended cells was formed, comprising the only "thallus" seen in these gametophytes throughout the experiment. From the small size of the cell mass, attained over a period of 4 months, it is unlikely that the lineage from each derivative of the end cell was more than a single cell (fig. 10).

The appearance of these retarded gametophytes is unlike that commonly described for gametophytes whose juvenile stage has been prolonged by subjection to a variety of other abnormal conditions, particularly those of light (4, 10). These latter filaments are almost always attenuated, branched, and usually produce antheridia. None of these characteristics was seen in the present gametophytes; they formed no sex organs, were never branched, and the cells, as already indicated, were not attenuated.

GAMETOPHYTES GROWN AT 10 MG./L.—These gametophytes showed two interesting types of peculiarities, the first of which concerns the position of the meristem. Although every gradation between the apical and lateral position of the meristem primordium is found among ferns with cordate prothallia (6), the prothallium of *P. longifolia* typically develops from a lateral meristem.

Development of the untreated gametophytes conformed to the usual type of

development of *P. longifolia* as described by GOEBEL (6, p. 204): "The surface of the prothallus which first develops from the germ-thread becomes at once the one wing, and the meristem which forms the vegetative point of the prothallus comes thereby to occupy a lateral position, and underneath it the second wing of the prothallus shoots out. Figure 150 exhibits this process in *Pteris longifolia*.[2] Here it will be seen that a one-layered cell surface is formed first of all from the germ-thread without the aid of an apical cell, and the anticlinal walls diverge at the apex. . . . . The intensity of the cell multiplication remains strongest at a lateral position on this cell surface, and there is the meristem in which often a two-sided apical cell is visible." In all the untreated plants observed in the present study, the meristem always arose laterally on the first formed wing (fig. 7).

Gametophytes treated with 10 mg./l., on the other hand, consistently formed their meristem apically. In this respect, these prothallia conformed to similar stages described for *Pteridium aquilinum* (14) and *Onoclea struthiopteris* (5, 14), in which the meristem typically lies in the longitudinal axis of the prothallium (fig. 11).

Because of the slow rate of cell division in treated gametophytes, the segments formed on either side of the meristem failed to extend beyond the vegetative point; thus, these prothallia did not become heart-shaped (fig. 15).

The second peculiarity was observed in prothallia 51 days old, on which a thick cushion consisting of distended, loosely arranged cells covered most of the ventral surface. In nearly all prothallia a prominent, compact tubercle was growing within the cushion close to the apex. A few prothallia had two tubercles lying side by side in the same forward position. In other prothallia these masses projected above the dorsal surface (fig. 15), but for the most part they were confined to the ventral surface.

The tubercles consisted of a large number of small, densely cytoplasmic cells with large nuclei (fig. 16). The cells did not exhibit any correlation but appeared to have divided at random. The entire cell mass was inclosed by the large vacuolate cells of the cushion.

Nearly all prothallia observed were without sex organs. Rarely, one or two unopened archegonia were seen on the cushion.

GAMETOPHYTES GROWN AT 1 MG./L.—Increase in size at this concentration also proceeded at a slower rate than in plain ZINZADZÉ's solution so that at the time of sex-organ formation these prothallia were considerably smaller than the control plants. Up to the 30th day, the gametophytes followed the usual pattern of development and attained the cordate shape. Subsequently, the cushion in the treated plants became more massive and extensive than that formed by the controls. Sex organs which later appeared on the cushion were more numerous on treated plants; especially antheridia.

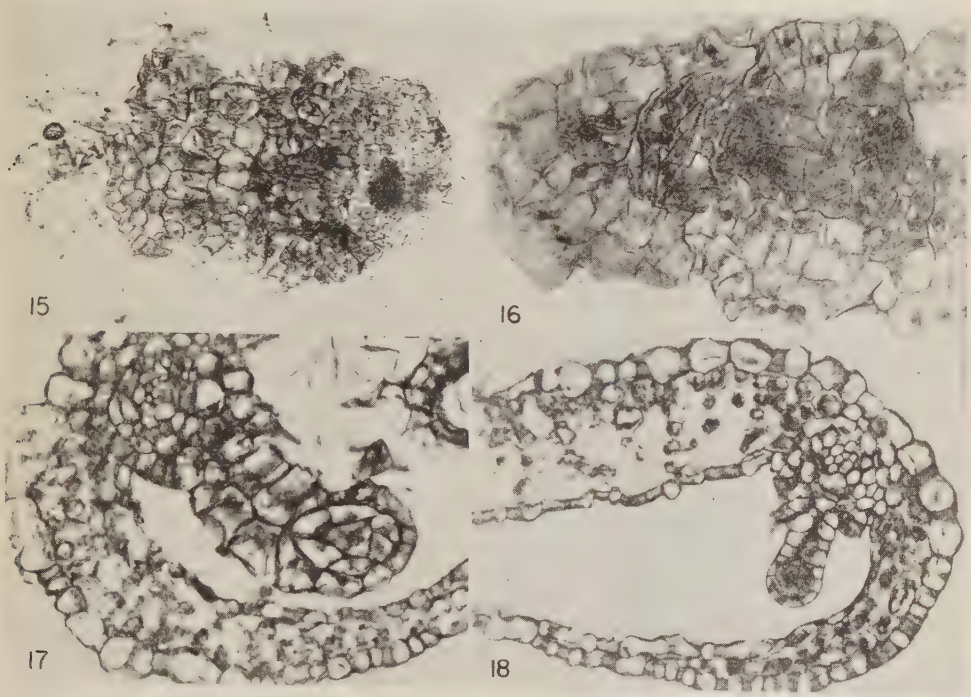
In plants treated at the 1 mg./l. concentration of ammonium 2,4-dichlorophenoxyacetate, there followed next renewed vegetative activity of the apical meristem, first evidenced by a small, tongue-like projection lying close to the base of the sinus formed by the two wings (fig. 14). Elongation of the projection proceeded in an upward direction accompanied by rapid lateral expansion. Thus, in 51-day-old gametophytes the meristem was borne at the apex of a third wing, usually fan-shaped with undulating margins (fig. 12), but some-

[2] Figure 7 of the present paper shows an equivalent stage to fig. 150 of Goebel.

times with margins curled to form a cylinder.

The vertical position of this wing permitted illumination on both sides so that it was not surprising to find sex organs on both surfaces. In addition, archegonia were present on the margin of the apex on either side of the meristem.

process were observed, but, by tracing cell derivatives, it is clear that marginal cells became meristematic, forming a plate directly, with no intervening filamentous stage (fig. 13). Owing to the simultaneous activity of many marginal cells, the adventitious thalli were crowded.



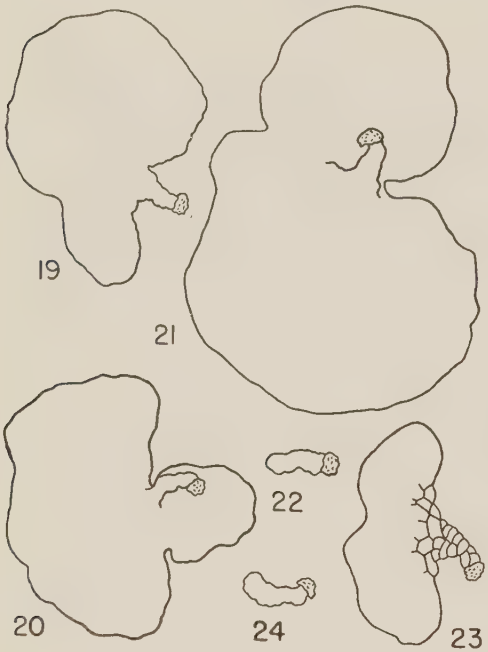
FIGS. 15–18.—Fig. 15, prothallium grown at 10 mg./l. solution of ammonium 2,4-dichlorophenoxyacetate, showing tubercle in apical region. Fig. 16, longisection of tubercle showing random arrangement of small, densely cytoplasmic cells. Fig. 17, transverse section of pinna treated with lanolin mixture of 500 p.p.m. 2,4-dichlorophenoxyacetic acid, showing grossly enlarged sporangium and receptacle. Fig. 18, pinna of control.

Simultaneously with the formation of the third wing in 51-day-old gametophytes, secondary prothallia were growing from the basal portion of the old wings (figs. 12, 13). Each of these new growths was intimately attached to the old gametophyte by means of a broad base, cells of which evidently were part of the parent tissue. Many of these older cells bore antheridia. No initial stages of this regeneration

Sex organs on plants treated with 1 mg./l. of ammonium 2,4-dichlorophenoxyacetate were functional, young sporophytes appearing on most prothallia in 120-day-old cultures. No irregularities were observed on primary leaves, and such plants were transplanted to agar in Petri dishes where they grew well for 4 months and gave every indication of continuing vigorous growth. In all respects these young sporophytes were

comparable to those formed at the same time on control prothallia.

TRANSPLANT EXPERIMENT.—Figures 19–24 show the results of experiments made to determine (*a*) the effect of 50 mg./l. of ammonium 2,4-dichlorophenoxyacetate on young prothallia not previously treated and (*b*) the permanency of the effect on gametophytes germinated and grown for a period of time on this same substance. The methods were the same as those previously described, except that the spores were sown on squares of glass cloth placed on the upper surface of inverted pots instead of directly on the pot surface. In this way prothallia were easily transferred from one pot to the other.



FIGS. 19–24.—Fig. 19, prothallium 21 days old grown from spore on plain nutrient solution. Fig. 20, prothallium grown for 21 days on plain nutrient solution, then transferred and grown for 12 days on 50 mg./l. of ammonium 2,4-dichlorophenoxyacetate. Fig. 21, prothallium grown continuously on plain nutrient solution for 33 days. Figs. 22–24, reciprocal of experiment shown in figs. 19–21.

Figure 19 shows a prothallium which was grown for 21 days on plain ZINZADZÉ's solution. A number of prothallia of this age were then transferred to the cultures containing 50 mg./l. of ammonium 2,4-dichlorophenoxyacetate. After 12 days these gametophytes (fig. 20) were compared with those of the same age—now 33 days old—which had not been transferred (fig. 21).

It is readily seen that the retardation of cell division is not a response in which the initial treatment of the germinating spore is a necessary condition but that the inhibitory action of ammonium 2,4-dichlorophenoxyacetate is similarly effective on later stages of prothallial development.

In the reciprocal experiment, spores were germinated and grown on cultures containing 50 mg./l. of the salt. After 21 days the minute filaments (fig. 22) were transferred to plain ZINZADZÉ's solution. Within 3 or 4 days the apex of the gametophytes had broadened considerably, and at the end of 12 days the two wings were well established (fig. 23). During this 12-day period the gametophytes which had remained in contact with the nutrient solution containing the growth-regulating substance showed no appreciable increase in size (fig. 24).

Renewed meristematic activity of transplanted prothallia was also observed in the tuberculate plants resulting from the 10 mg./l. treatment. A number of these gametophytes were transplanted to agar plates in an effort to induce further growth of the tubercles. Instead, germinal filaments soon emerged from marginal and inner cells of the thallus. These filaments formed the usual spatulate lobe, developed a meristem, and became cordate. The original prothallia died.

It is apparent from the regenerative growth of the transplanted prothallia

that the inhibitory activity of ammonium 2,4-dichlorophenoxyacetate does not persist once the plants are removed from actual contact with the effective solution. This behavior is not wholly unexpected when one considers that each cell of the thallus is potentially capable of reproducing a new prothallium and that the amount of the substance in a single cell at any one time must be exceedingly small. The derivatives of such a cell, having once succeeded in dividing, would then rapidly become removed from the influence of the residual substance.

Sporophyte

Applications of lanolin mixtures and aqueous solutions were made at the basal portion of the petioles, usually below the first pinna, at the time the leaves had unfolded about one-half their ultimate length. The mature plants at this time were producing leaves 40 cm. or more in length, while the leaves of the young potted plants were rarely 15 cm. long. Applications on the mature plants were at 500 and 1000 p.p.m.; the young plants, in addition to these concentrations, were treated with 125, 250, and 2000 p.p.m.

Gross reactions.—Within 24–48 hours after treatment the petioles at various distances from the point of application showed pronounced downward bending, except at a concentration of 125 p.p.m. at which there was no reaction. As the croziers slowly unrolled, the petioles continued bending in one direction or another until the full length of the leaf was attained. In all cases the final length was less than that of the controls.

Reactions of the pinnae were primarily characterized by a failure to unfold completely (fig. 25*d*). For a period of 3 or 4 weeks after treatment the pinnae slowly continued to unroll and expand, after which they persisted in one or another of the stages of unfolding. Retardation in this respect was greatest in the uppermost (i.e., the youngest) pinnae and decreased basipetally to the pinnae which were fully expanded at the time of treatment. These latter pinnae exhibited no effects from the treatments.

Intensity of responses appeared to be related more to the relative age of leaves at the time of application than to concentrations used. In preliminary experiments numerous applications were made on leaves of similar ages, and the intensity of responses showed considerable variation in which the most marked reactions were not always associated with the highest concentrations used (figs. 25*a*, *b*, *c*). Responses were more rapid to aqueous solutions than to the lanolin mixtures.

Histological reactions.—Several days after the curvatures of the petioles appeared, the leaves became unusually inflexible, the young pinnae and young portions of the petioles acquiring a brittle quality. Although mature petioles of *P. longifolia* possess a natural toughness because of the thick-walled epidermis and the several layers of thickened outer cortical cells, newly differentiated parts of the petiole are supple.

Transverse sections of these young parts showed that the subepidermal layers of the cortex were already considerably thickened, hence more mature than comparable tissues of the control plants. Tertiary thickening of the metaxylem elements also occurred earlier in the treated plants, but this was evident only in a small number of elements surrounding the invaginated phloem in the arms of the horseshoe-shaped stele. The most pronounced thickening of the vascular elements occurred in the treated basal portions of petioles of mature

plants; especially where the aqueous solution of ammonium 2,4-dichlorophenoxyacetate was used.

Aside from the early maturation of these tissues, and a slightly greater thickening of all the cortical and stelar tissues, no other histological irregularities of the petioles were evident. There were no secondary outgrowths, tumors, proliferations, or derangements in the position of the tissues.

THE SPOROPHYLL.—The terminal pinnae, from which the observations of the reactions of the sporangium were made, were treated when the pinnae were completely unfolded and the indusium was still closed. By the use of a hypodermic syringe, a thin, uniform thread of the lanolin mixture of the acid (500 p.p.m.) was laid along the indusium on one side of the pinna. The indusium on the other side received no treatment. On a second pinna a thin band of the aqueous solution of ammonium 2,4-dichlorophenoxyacetate (500 p.p.m.) was painted on one indusium with a camel's-hair brush. These treatments were repeated on several plants.

Within 2 days after application there was a marked epinasty of the treated

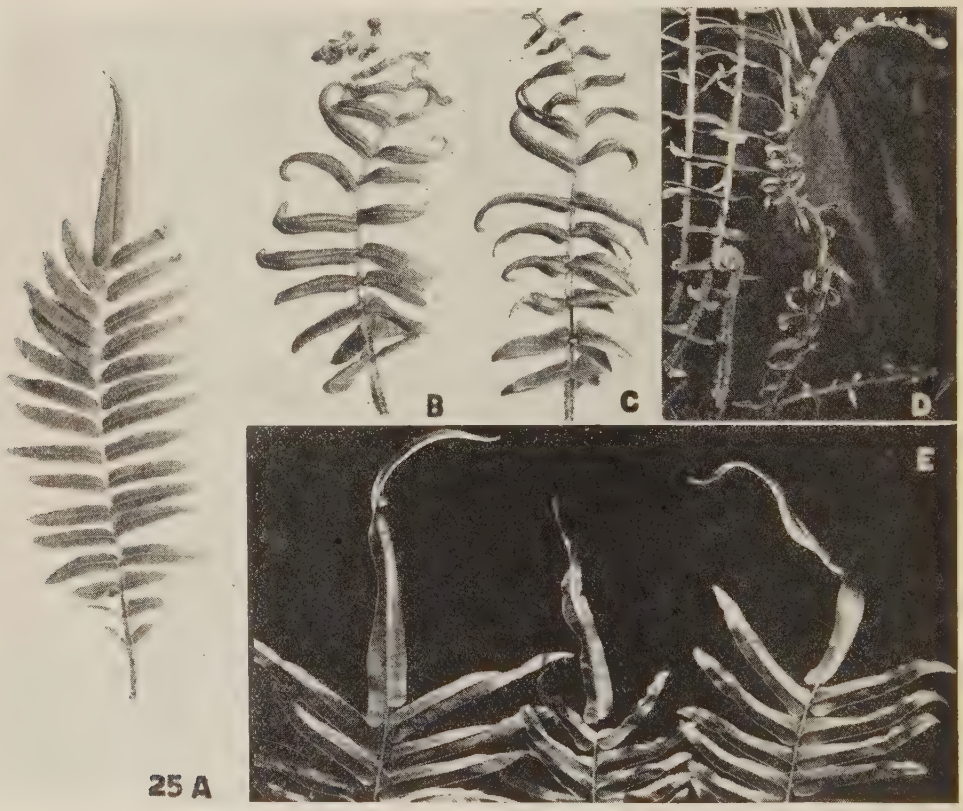


FIG. 25.—Fig. 25*A–D*, leaves 30 days after treatment with ammonium 2,4-dichlorophenoxyacetate: *A*, control; *B*, 500 p.p.m.; *C*, 2000 p.p.m.; *D*, 1000 p.p.m., leaf of mature plant showing marked failure of pinnae to unroll. Fig. 25*E*, terminal pinnae 14 days after unilateral treatment: *left*, lanolin mixture of 500 p.p.m. 2,4-dichlorophenoxyacetic acid; *middle*, lanolin control; *right*, aqueous solution of 500 p.p.m. ammonium 2,4-dichlorophenoxyacetate.

halves of the pinnae. By the 4th day the pinnae treated with the aqueous solution showed a spiral twisting along the axis of the treated indusium. Similar but less pronounced responses followed the application of the lanolin mixture. In preliminary experiments pinnae so treated persisted in the twisted condition for an indefinite time without further gross responses. The present material, however, was collected at the end of 14 days (fig. 25*e*).

Histologically, the pinnae were little affected by the growth-regulating substance. Transections showed enlargement of cells of the upper epidermis associated with the curvatures on the treated side of the blade, and there was also some derangement of the palisade layer accompanying this same response.

In contrast to the inactivity of the pinna in general, the quantitative reaction of the sporangia and receptacle was very striking. It was characterized by pronounced cell enlargement which resulted in the growth of these structures to more than twice their usual size.

The receptacle of *P. longifolia* is not prominent, being only slightly elevated above the plane of the lower epidermis. The receptacle beneath the treated indusium, however, became a rather massive structure which protruded well beyond the epidermis (fig. 17). The receptacle on the untreated side of the pinna was relatively unaffected (fig. 18).

A comparison of figures 17 and 18 also shows that the sporangia beneath the treated indusium were markedly affected and that the cells of these sporangia, particularly those of the stalk and tapetum, exhibited the same swollen condition as the receptacular cells. Although the sporangia and receptacles are meristematic up to the time the indusium is fully open and the sporangia are mature, these organs were not stimulated to proliferate. The number of cells comprising the receptacle, and especially the sporangia, were always consistent with the particular stage of development in which they were observed.

Discussion

The results of the experiments on the gametophytes show that the primary and general effect of ammonium 2,4-dichlorophenoxyacetate was inhibition of growth. This effect was essentially the result of the retardation of cell division with little or no effect upon cell enlargement. In contrast to the behavior of the sporophytes, the quantitative responses of the prothallia were directly correlated with the concentration of the effective substance. Thus, at .50 mg./l. retardation was so great that no plate was formed, whereas at 1 mg./l. the gametophyte developed into a heart-shaped prothallium with functional sex organs.

Aside from the obvious inhibitory effect on growth of the gametophytes, it is less apparent whether the unusual structures observed on the prothallia were the result of the direct influence of ammonium 2,4-dichlorophenoxyacetate or whether these growths were secondary responses resulting from other causes.

For example, it is not certain whether the tubercles which appeared on prothallia grown at 10 mg./l. were apogamous embryos whose differentiation was unorganized due to the influence of the growth-regulating substance or whether they were gall-like masses resulting from proliferation of the cushion cells.

Heim (8) has described certain projections from the cushion of *Doodya caudata* from which apogamous sporophytes arose, and the internal appearance of these structures as figured by him bear a resemblance to that of the present tuber-

cles. In the case of *Doodya*, however, the sporophytic nature of the projections was confirmed at a relatively early stage by the development of tracheids, later followed by the formation of the apices of the leaf, stem, and root of the embryo, which then grew in a normal manner.

Usual evidences of apogamy in ferns (15), such as the formation of tracheids, or hairs surrounding the embryo, were not present in the treated prothallia, and the cell masses as seen in longitudinal and transverse sections were not recognizable embryos. In addition, transplants made to induce further growth of the tubercles failed. It does not appear likely that the tubercles were sporophytic.

The renewed activity of the apex of prothallia grown at 1 mg./l. also suggests the possibility of secondary formative influences. It is well known that the gametophytes of the leptosporangiate ferns are much more plastic than the sporophytes and that relatively minor alterations of the environment of the prothallia induce profound form changes.

In this connection it is interesting that the outgrowth of the apex in the treated prothallia is strikingly similar to that obtained by ALBAUM (1) in young prothallia which were changed from their nearly vertical position to a horizontal one. Following the reorientation, the apex in these prothallia began to grow upward toward the source of light, and there was ultimately formed a third wing similar to that shown in figure 12. It is not to be inferred, however, that the apical outgrowths in the treated prothallia necessarily originated in this same manner, since their position relative to the source of light was not experimentally altered.

The curvatures of petioles and pinnae following treatment of the leaves of *P. longifolia* indicate that the reactions of the sporophyte of this fern to 2,4-dichlorophenoxyacetic acid are not markedly different from those of various angiosperms that have been similarly treated.

In the first report of the activity of this substance on plants, ZIMMERMAN and HITCHCOCK (17) designated as a general type of response "the negative curvatures of stems and leaves, i.e., responses involving cell elongation." Subsequent studies by these investigators and others have shown that distorted growth of stems and leaves is readily induced in many angiosperms by applications of 2,4-dichlorophenoxyacetic acid and is one of the first conspicuous effects of such treatments. In the same paper, ZIMMERMAN and HITCHCOCK described a second type of effect—"morphogenetic activity." This term was used to refer to "modifications of organs which involved responses other than curvatures due to cell elongation." Except for the modification of sporangia, which was basically the result of cell enlargement, this second type of response was strikingly absent in the treated sporophytes. The tissues of the leaves were relatively unaffected by the applications of 2,4-dichlorophenoxyacetic acid or its ammonium salt and were quite insensitive to the formative influences of this substance. The resistance of the sporophyte of *P. longifolia* to histological modification when treated with 2,4-dichlorophenoxyacetic acid is not unique but serves as another example emphasizing the selective action of this substance.

Summary

1. Spores of *Pteris longifolia* were germinated and the gametophytes were grown uninterruptedly with ZINZADZÉ's

solution containing 0, 1, 10, and 50 mg./l. of ammonium 2,4-dichlorophenoxyacetate.

2. Spore germination was not adversely affected either in time or in percentage by the presence of the growth-regulating substance.

3. The smaller size of treated gametophytes was due to the smaller number of cells comprising them and not to the size of the cells. The intensity of retardation of cell division was proportional to the concentration of the ammonium salt used.

4. Treatment at 10 mg./l. resulted in the formation of the meristem primordium in the apical instead of the usual lateral position. These prothallia did not become heart-shaped and rarely produced sex organs.

5. Fifty-one-day-old gametophytes growing at 10 mg./l. exhibited tubercles consisting of small, densely cytoplasmic cells in random division within the massive cushions.

6. Prothallia growing at 1 mg./l. showed renewed vegetative activity of the meristem to form a third wing. Simultaneously, secondary prothallia were growing from the basal portion of the old prothallial wings. Functional sex organs were more abundant on the prothallia grown at this concentration than on the controls.

7. Young prothallia grown for 21 days on plain ZINZADZÉ's solution made no appreciable growth after being transferred to a culture containing 50 mg./l. of ammonium 2,4-dichlorophenoxyacetate. Conversely, gametophytes which were germinated and grown for 21 days on the latter culture showed a rapid recovery to normal growth after being transferred to plain ZINZADZÉ's solution. Tuberculate prothallia transferred from 10 mg./l. cultures to agar plates quickly produced secondary prothallia which were normal in all respects. The tubercles made no further growth.

8. Leaves of young and old sporophytes of *P. longifolia* were treated with lanolin mixtures of 2,4-dichlorophenoxyacetic acid (500 p.p.m.) and aqueous solutions of the ammonium salt of the acid (125–2000 p.p.m.). The petioles showed marked curvatures and the unrolling of pinnae was retarded. These responses were permanent. There was no reaction from applications of 125 p.p.m.

9. Histologically, the applications resulted in earlier maturation of the petioles, especially in the outer cortex and the metaxylem elements surrounding the invaginated phloem. There were no tumors, proliferations, or derangement of the tissues. The responses were more marked where the aqueous solutions were used.

10. Unilateral treatment (500 p.p.m.) of terminal pinnae resulted in epinasty and spiral twisting of these organs. Beneath the treated indusium, the receptacle and sporangia were grossly enlarged due to cellular distension. There was no unusual increase in the number of cells comprising these structures.

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